THE FERMENTATION OF POLYSACCHARIDS BY BACILLUS AEROGENES

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From recent work on the coli-like bacteria it is agreed that they may be divided into two subgroups which are differentiated by the Voges-Proskauer or methyl red reactions. These two groups are quite well correlated with habitat. The B. coli group (Voges-Proskauer negative — methyl red positive) is characteristically of fecal origin, whereas the B. aerogenes-cloaca group (Voges-Proskauer positive — methyl red negative) is relatively infrequent in feces, but predominates in the soil and on grains. B. aerogenes, although rarely found in the feces as voided, is a common inhabitant of the upper part of the intestinal tract of man. Under certain conditions it may constitute a considerable portion of the coli-like bacteria of a stool. It thus becomes of some practical significance to differentiate the B. aerogenes of intestinal origin from the B. aerogenes found in soil and on grain. Rogers pointed out that the fermentation of adonitol may be employed for this purpose with a high degree of reliability. It was thought that a study of the fermentation of the polysaccharids from different sources, particularly glycogens from vegetable and animal sources might possibly be of value in differentiating fecal from nonfecal types of B. aerogenes.

HISTORICAL

In 1883 Wortman was led to believe that starch could be decomposed by bacteria, and he observed that if starch was prepared and allowed to ferment spontaneously an organism which he termed Bacterium termo predominated. This work led him to infer that many bacteria produce an enzyme which shows the properties of diastase. He also observed that various starches were not broken down with equal celerity and suggested that this is due to differences in specific gravity.

Durham (1901) states that members of the B. lactis-aerogenes group may be separated from other coli-like bacteria by their ability to ferment starch and inulin, and thinks that they may be separated into three groups by their action on these substances. Some ferment starch and inulin with gas and acid production, others starch alone and still others inulin alone.

F. A. Baldwin (1917), in a preliminary report of work on the colon group, employing the Bergey and Deehan classification, finds that many forms may be differentiated by the differences in their action on corn and potato starch.

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Johnson and Levine observed that the B. aerogenes types decomposed corn starch quite readily. About 18% of their strains formed acetyl-methyl-carbinol whereas the others apparently did not, indicating the possibility of differentiation within the group.

It is difficult to review the literature on starch fermentation by bacteria because it is very rarely stated what starch was employed. The number of recorded instances in which starches from different sources were used is negligible.

EXPERIMENTAL

Sources of cultures.—In the initial experiments, 117 strains were employed, including 49 of the B. aerogenes type which were starch fermenters and 68 different varieties of B. coli and B. cloacae; the latter being regarded as starch nonfermenters. It was observed that the strains of B. coli and B. cloacae did not ferment any of the polysaccharids employed with acid and gas production and will therefore not be discussed further.

TABLE 1

AMONG THE 49 B. AEROGENES STRAINS INCLUDED

Soil	13
Sewage	6
American Museum of Natural History	12
Grains (furnished by L. A. Rogers)	3
Lederle Laboratories	1
University of Toronto (originally from the Pasteur Institute, Paris)	1
Isolated from virus hogs, Iowa State College	8
American Museum (marked B. mucosus-capsulatus)	2
Chicago University (B. mucosus-capsulatus)	1
Northwestern Medical School (B. mucosus-capsulatus)	1
University of Toronto (B. pneumoniae-Friedländer)	1

Except as indicated the original sources of these strains were not known.

Medium.—The medium used was made up as follows: A solution of 1% Witte's peptone and 0.5% dipotassium phosphate in distilled water was prepared, flasked and autoclaved. A quantity sufficient for the entire work was made up at one time.

Preparation of Test Substances.—Eight hundred c c of the dipotassium phosphate-peptone solution were brought to a boil and while heating 5 gm. of the test substance were thoroughly mixed in 200 c c of the cold medium and added slowly to the 800 c c of the boiling medium. The mixture was then boiled for ten minutes and the loss of weight made up with distilled water. This gave a medium containing 0.5% of the test substance. The medium was then placed in Durham fermentation tubes, autoclaved for 10 minutes at 10 lbs. pressure and quickly chilled with cold water. When prepared in this manner there was no evidence of the presence of reducing sugars when tested with Fehling's solution.

The following test substances were used:

Starches.—Arrowroot-Post Natal (*Maranta sp.*)
Arrowroot-St. Vincent's (*Maranta sp.*)
Barley (*Hordeum sativum Jess.*)
Bean (*Phaseolus sp.*)
Buckwheat (*Fagopyrum esculentum*)
Canna (*Canna edulis Edw.* and other species)
Corn (*Zea Mays L.*)

Ginger (*Zingiber officinalis Roscoe*)
Lentil (*Lens esculenta Moench*)
Oat (*Avena sativa L.*)
Pea (*Pisum sativum L.*)
Potato (*Solanum tuberosum L.*)
Rice (*Oryza sativa L.*)
Rye (*Secale cereale L.*)
Sago (*Cycas sp.*)
Tapioca (*Manihot utilissima Pohl*)
Wheat (*Triticum sativum Lam.*)

These starches were all "Lilly's Authentic Starches," prepared by the Eli Lilly Co. They were given a careful microscopic examination and appeared to be pure.

Inulin.—Merck's Inulin-Kiliani was used.

Glycogen.—Horse glycogen, from the liver of a normal horse, was prepared by Pflüger's method as given by Plimmer. Polyporus (*Polyporus rulphureus*) was used as an example of a plant glycogen. The latter specimen was prepared by the Chemical Section of the Agricultural Experiment Station through the courtesy of Dr. Dox.

Hemicellulose.—Hemicellulose from date seeds was used.

Incubation.—Incubation was at 37.5 C. for one week. For the first 3 days the tubes were looked over each day, and those that did not show gas were shaken to insure the entrance of the organisms into the gas tubes.

Records.—At the end of three days a preliminary record of gas production was made. At the end of a week the final records were made, consisting of the approximate percent. of gas produced and the reaction to methyl red.

Results.—About half of the cultures were tested for their reaction to phenolphthalein. The hydrogen-ion concentration was such that all were acid or neutral to phenolphthalein.

Fehling's solution and Barfoed's reagent were used in testing for reducing sugars. None of the tubes tested showed the presence of reducing sugars.

The Voges-Proskauer reaction was also tried on half of the strains and was found to be uniformly negative.

Forty-six strains produced gas from all the starches studied. Of the three organisms remaining, a B. aerogenes isolated from virus hog failed to form gas from bean and ginger starch, a B. pneumoniae Friedländer culture from the U. S. Hygienic Laboratory, failed to produce gas from lentil, oat and wheat starch, and the Roger's strain produced small amounts of gas from corn, tapioca and wheat starch only.

Only three organisms produced gas from inulin. These included two strains of B. aerogenes from soil and the culture of B. lactis-aerogenes from the University of Toronto.

Three organisms (all B. aerogenes types from soil) produced gas from the two glycogens. Two of the three inulin fermenters were included in this group of organisms. The glycogen fermenters produced gas from all the starches.

Twenty organisms produced gas from dulcitol. Eight of these were indol negative and twelve indol positive. As reported by Johnson and Levine, there appeared to be no correlation between the fermentation of dulcitol and indol production.

None of the organisms used produced gas from date seed hemicellulose.

DISCUSSION

Gas Production.—It was observed that if an organism fermented one starch it usually fermented all the starches tested. Of the 49 strains studied there were only 3 that did not conform to this rule, and these 3 did not seem to be interrelated with reference to the starches from which they produced gas. This peculiar behavior of a few of the strains toward starch is difficult to explain.

A study of starch from the chemical and physical standpoint has led to the belief that it is not a uniform compound, but exists in many isomeric and polymeric forms in different plants. Reichert, in discussing stereochemistry and some of its applications, says:

> (1) That it is theoretically possible for a complex compound, such as starch or hemoglobin, to exist in a countless number of stereoisomeric forms; (2) that the slightest alteration in the configuration or arrangement of the component units of a molecule may give rise to a change of properties that may be profound, and sometimes of a predictable character; (3) that stereochemistry is inseparably associated with the problems of nutrition, species, disease, heredity and the innumerable manifestations of protoplasmic activity which in the aggregate constitute life.

Fischer emphasizes the fact that stereoisomeric substances often show a greater differentiation in their properties than is usually observed in related isomers.

Isomeric substances, such as the carbohydrates, are unique in their fermentation reactions in that each one requires a specific enzyme to bring about its decomposition. One enzyme is often capable of breaking down all the members of a group which do not bear an isomeric relation to each other. The fats, for example, while differing widely in molecular weight, belong to a homologous series, and one enzyme, lipase, can cause the decomposition of all the members of the group. Fischer believes that it is necessary for an enzyme to have a complementary configuration in order to bring about the decomposition of a compound. To use his analogy, the enzyme must be adjusted to the substance, much as the key is adjusted to the lock.

A brief glance at the literature is sufficient to show that bacteria that are quite closely related show wide differences in the enzymes produced and in the consequent fermentations.

The fact that an organism which ferments one starch usually ferments all the others would indicate that the same enzyme is capable of breaking down all of them and that the starches are polymers. Another point of view would be that the organism produced a multiplicity of enzymes.

Acid Production.—The most striking thing noted in this work was that although organisms fermented all the starches with gas production there was a very marked difference in the hydrogen-ion concentration produced with different starches. The following table summarizes the reactions to methyl red of the organisms with the starches which they fermented. It was not possible, at the time this work was being done, to repeat the experiments in the case of a few contaminated cultures. There are, therefore, several differences in the total number of fermentations of the several starches recorded in this table.

TABLE 2

REACTIONS TO METHYL RED OF ORGANISMS AND STARCHES THEY FERMENTED

Starches	Acid	Neutral	Alkaline
Arrowroot P. N.	15	5	29
Arrowroot S. V.	16	14	19
Barley	10	5	33
Bean	10	5	34
Buckwheat	8	2	39
Canna	22	4	21
Corn	13	6	30
Ginger	3	4	41
Lentil	7	3	38
Oat	0	2	46
Pea	17	7	25
Potato	19	3	27
Rice	4	5	29
Rye	14	5	30
Sago	20	6	23
Tapioca	20	5	24
Wheat	12	6	31

The differences in reaction with the same organism on different starches and with different organisms on the same starch were so varied that correlation tables seemed of no practical value for the differentiation of strains.

These differences are of considerable interest, at least from the theoretical standpoint. It is customary to say that when a starch is decomposed that it has been hydrolyzed, and it is usually assumed that the decomposition has taken place through a number of stages; i. e., erythrodextrin, achroadextrin, maltose and glucose. In bacterial fermentations the glucose is broken down with the liberation of acid, gas and other end products usually observed. In this group of organisms we are dealing with strains which assimilate glucose with the production of acetyl-methyl-carbinol and in a concentration of 0.5% glucose give an alkaline reaction to methyl red. It was shown by Levine, Weldin and Johnson that with a concentration of 1.0% glucose the reaction to methyl red becomes alkaline after 3 days at 37 C. If

it is assumed that all the starch is hydrolyzed to glucose the maximum concentration would be 0.556% glucose, a concentration at which all the B. aerogenes strains would give an alkaline reaction after seven days at 37 C. The fact that in many instances these organisms produced an acid or neutral reaction seems to indicate that the starches are not utilized as glucose. If the starches are first reduced to glucose before utilization we should expect to find the same end products as when glucose is used directly.

It is generally recognized and accepted that B. aerogenes characteristically produces acetyl-methyl-carbinol from glucose. This was the case with the cultures used in this study. Of the 25 strains tested for acetyl-methyl-carbinol from starch fermentation not a single strain was positive, which again indicates that the decomposition of starch probably did not progress to the glucose stage before assimilation by the bacteria.

In no instance was there any evidence of a reducing sugar when tested with Fehling's solution or Barfoed's reagent. This may be explained on the assumption that (1) no reducing sugar was formed, or (2) reducing sugar was produced in small quantities and quickly utilized. If the latter were true, we would expect to find the same end-products as when glucose is used directly. The findings tend to show that in the decomposition of a starch by bacteria there is some metabolic process going on which is quite at variance with accepted ideas. An investigation of what really takes place under these conditions will, at least, be of considerable theoretical interest.

SUMMARY

B. aerogenes usually ferments starches of widely different origins.

The results obtained tend to show that B. aerogenes does not hydrolyze starch through the usual series of compounds and eventually utilize it as glucose.

The difference in the amount of gas and the final reaction produced when a given strain of B. aerogenes ferments starches from different sources indicates that there is some difference in the composition of starches from different sources.

Starches do not appear to be of value in the differentiation of strains of B. aerogenes derived from fecal and nonfecal sources.